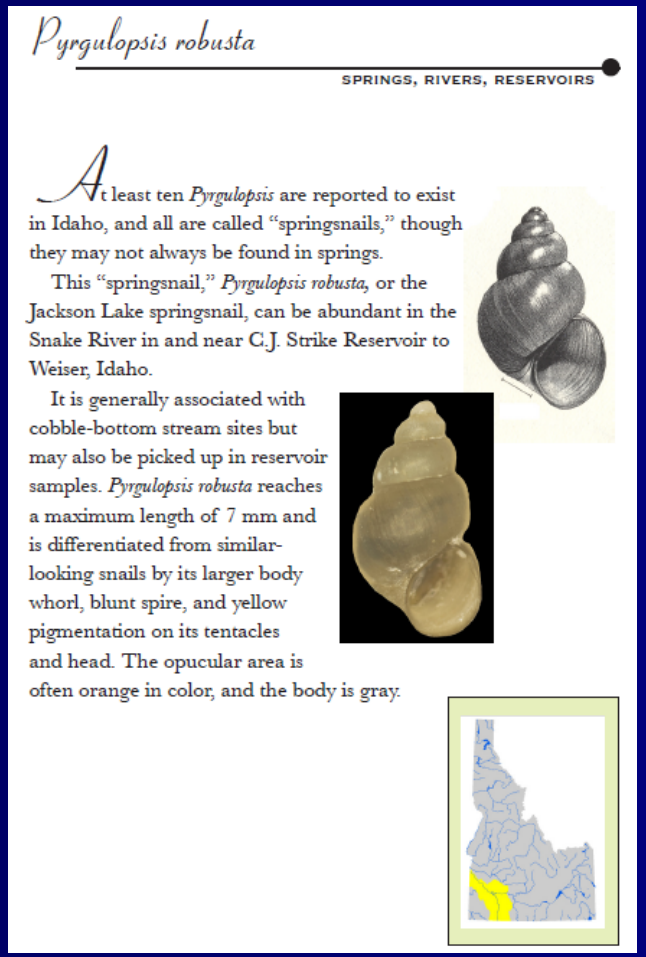
EPA Support for Science Supporting Environmental Decisions

- EPA support for regions that produce, maintain or use science information either directly or indirectly in the fulfillment of their missions
  - Regional Applied Research Effort (RARE) grants
  - Regional Methods (RM) program
- Both grant programs are competitive nationally
- Both programs have a regional sponsor, an EPA ORD research partner, and possibly one or more other research partners

RARE and RM Grants with USGS-Columbia

- 2006 – 2009 RARE grant: Developing a Method for Propagation of Endangered Snails for Use in Toxicity Testing
- 2009 – 2011 RM grant: Development of Methods for Propagation of ESA-Listed and Surrogate Species of Freshwater Snails for Use in Toxicity Testing
- 2011 – 2013 RM grant: Toxicity Estimation in Threatened and Endangered Species: Fairy Shrimp

Idaho Springsnail (*Pyrgulopsis idahoensis*)



Pyrgulopsis robusta

SPRINGS, RIVERS, RESERVOIRS

At least ten *Pyrgulopsis* are reported to exist in Idaho, and all are called "springsnails," though they may not always be found in springs.

This "springsnail," *Pyrgulopsis robusta*, or the Jackson Lake springsnail, can be abundant in the Snake River in and near C.J. Strike Reservoir to Weiser, Idaho.

It is generally associated with cobble-bottom stream sites but may also be picked up in reservoir samples. *Pyrgulopsis robusta* reaches a maximum length of 7 mm and is differentiated from similar-looking snails by its larger body whorl, blunt spire, and yellow pigmentation on its tentacles and head. The opucular area is often orange in color, and the body is gray.

- **First species tested in this work**
- **Found to reproduce well under laboratory conditions**
- **Federally endangered species when work started**
- **Taxonomic revision after work completed merged several springsnail species into the Jackson Lake springsnail (*P. robusta*)**
- **Delisted from ESA after taxonomic revision**

Bliss Rapids Snail (*Taylorconcha serpenticola*)

Taylorconcha serpenticola

SPRINGS, TRIBUTARIES, RIVERS

Taylorconcha serpenticola is one of two similar species in Idaho named in honor of Idaho malacologist Dwight Taylor. This snail is small; about 2-3 mm full grown and is about as wide as high.

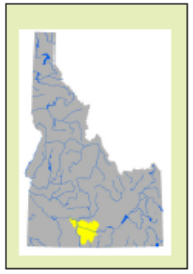
It has two color morphs: a blonde morph and an orange morph, though variation exists in the color of both varieties.

This snail is found in the Snake River and associated springs between Twin Falls and Glens Ferry. It is commonly known as the Bliss Rapids snail, and lives on the bottoms of cobbles; almost never on the sides or tops.

This species needs cool, flowing water to survive, and isn't found in any of Snake River reservoirs. This snail is federally protected as a threatened species.

Please do not collect it!

Bliss Rapids snail (BR)



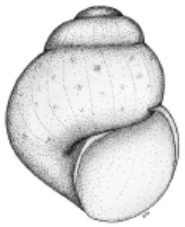
- Second species tested in this work
- Species of major concern to FWS
- Does not reproduce well under laboratory conditions (at least not yet)
- Federally threatened species

Pebblesnails (*Fluminicola spp.*)

Fluminicola spp.

SPRINGS, TRIBUTARIES, RIVERS

This genus, which contains about a dozen species, belongs to the largest family of freshwater snails in Idaho, the *Hydrobiidae*. Another large genus, the Pyrgs, contains about ten species.

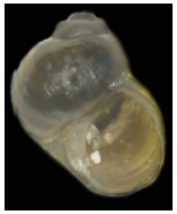


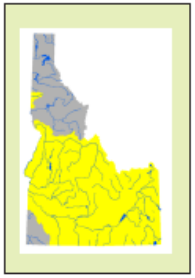
ETHAN NEDEAU

All of the species in this genus are called "pebblesnails" because they look like pebbles on stream bottoms.

At maturity, these snails will be about 7-10 mm. Pebblesnail shells are black and often have a worn spire that appears white. The animal is dark grey to black, and they can be difficult to distinguish from other *Hydrobiidae* such as *P. bruneauensis* or *T. serpenticola* except by their size and relative abundance.

Often the most abundant snail in rivers, springs, and tributaries, they are largely absent from reservoirs. These snails are found in Idaho in the Snake River from the Henry's Fork to the Oregon border and in many tributaries (including the Wood, Bruneau, and Portnuef Rivers) and springs. *Fluminicola* are common in the western United States. Their taxonomy is currently under revision.



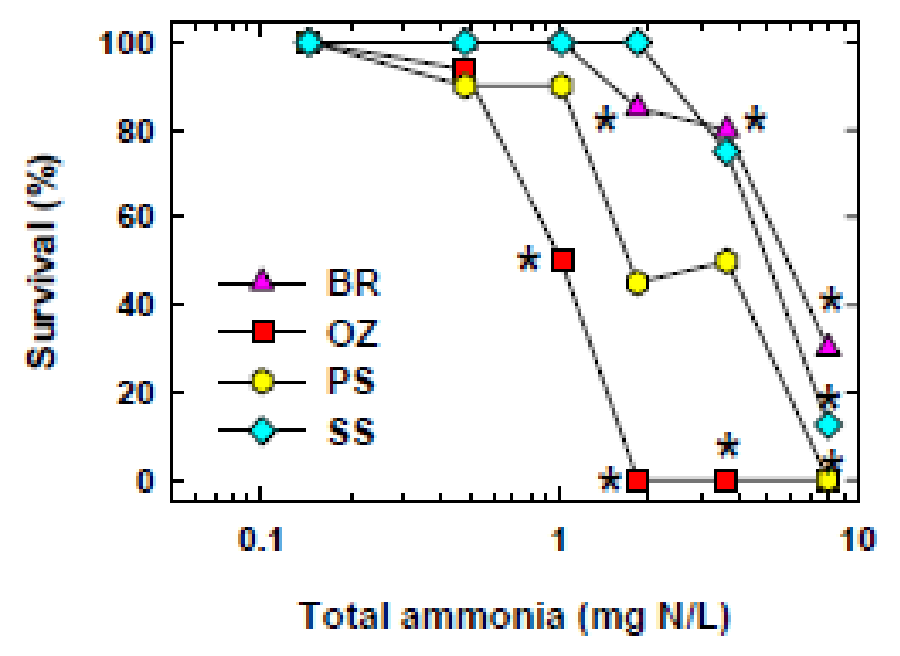


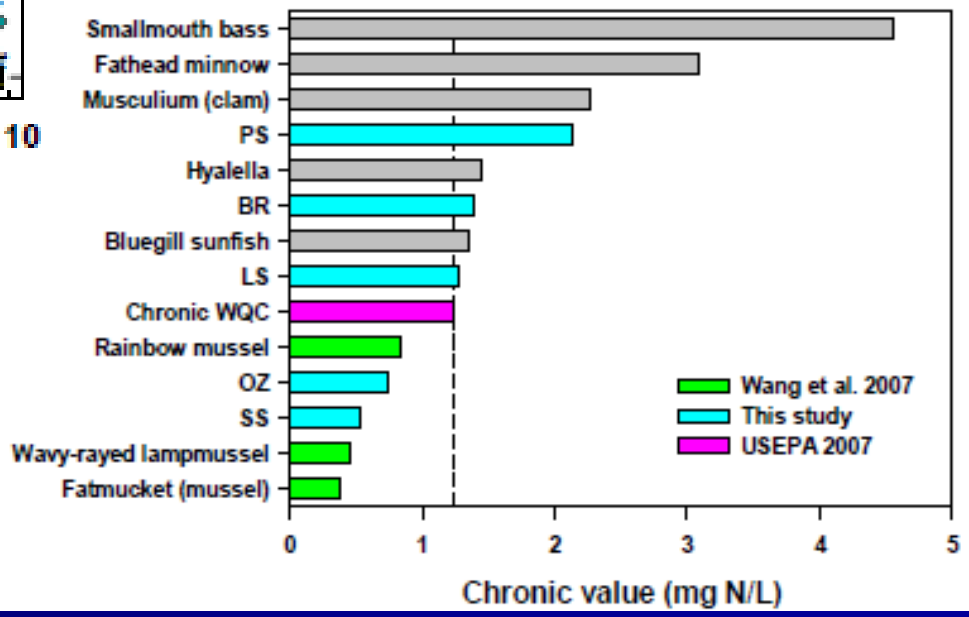
- Third Idaho species tested in this work
- Taxonomy not well defined yet in this species complex of 10 or more species
- Reproduce moderately well under laboratory conditions

Toxicity Test and Culturing Methods

1. Test organisms	See Table 2
2. Test type	Flow-through toxicity test
3. Test Duration	28 d
4. Toxicants	Copper (copper sulfate) Pentachlorophenol (stock prepared in triethylene glycol) Ammonia (Ammonium chloride; pH of stock adjusted to 8.3 with sodium hydroxide)
5. Dilution series	Control and five concentrations in 50% dilution series (see Tables 3-5 for nominal and measured concentrations)
6. Temperature	20±1°C
7. Lighting	Ambient laboratory light (about 200 lux, 16 hr light:8 hr dark)
8. Aeration	None
9. Feeding	Homogenized flake fish food suspension (Tetrafin®, 6 mg/ml): Pondsnails (and adults of other species), 1 ml/beaker/d; Idaho springsnails, 0.5 ml /beaker on Monday, Wednesday, and Friday (daily starting on day 14).

Results – Ammonia 28-day chronic





Results of 28-day Chronic NH_3 Retest with Pebblesnails

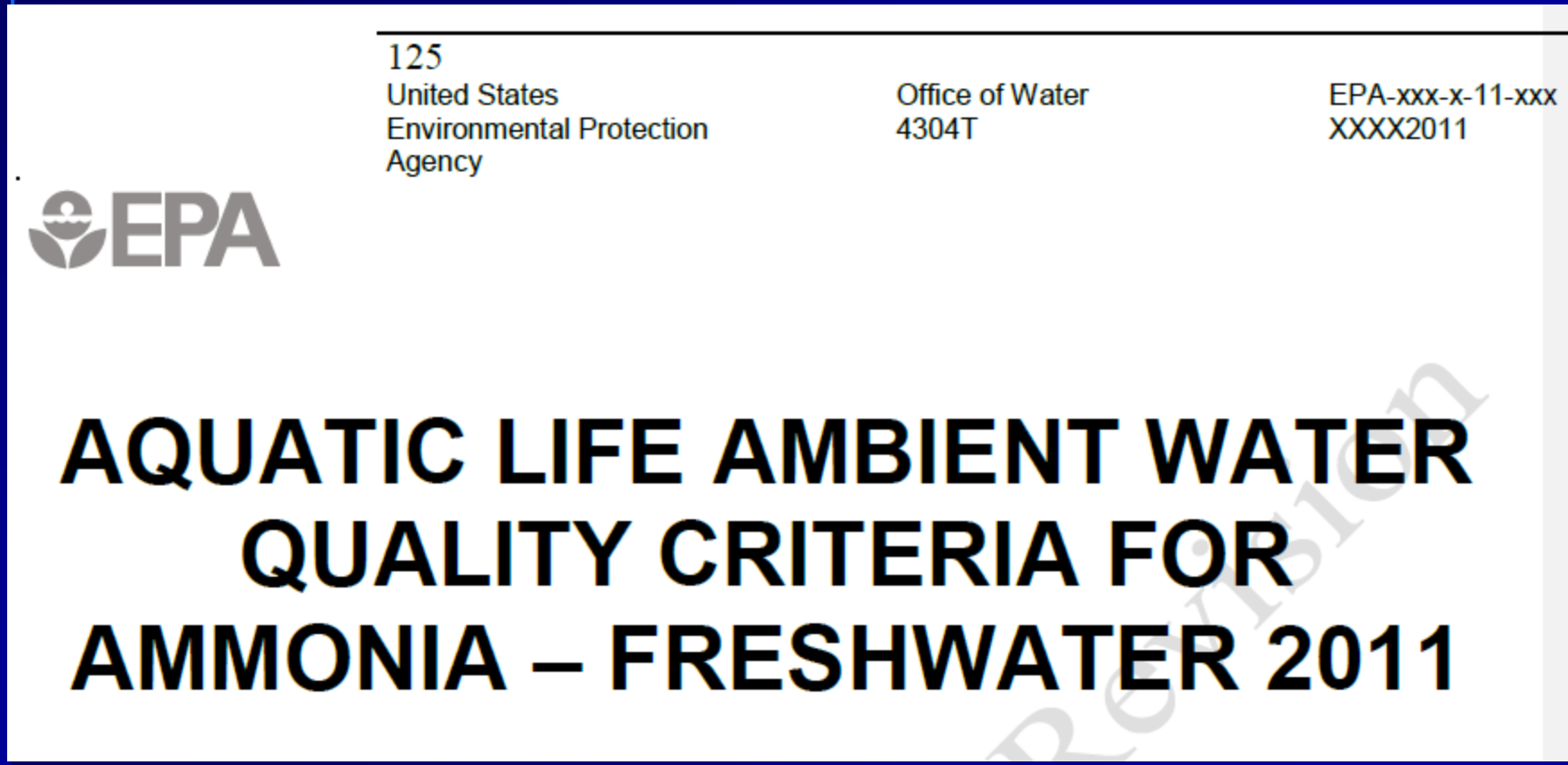
Large snails (Reps 5-8; mean starting shell length 1.8075)

Total Ammonia (mg N/L)		Survival (of 10): p<0.0001				Length (mm): p=0.18		
Nominal	Measured	N	Mean	Std. Error		N	Mean	Std. Error
Control	0.12	4	9.25	0.48		4	2.3572	0.0553
0.5	0.57	4	10.00	0.00		4	2.2923	0.0835
1.0	0.97	4	9.75	0.25		4	2.4014	0.0777
2.0	2.06	4	10.00	0.00		4	2.2679	0.0486
4.0	3.67	4	1.00	0.71	*	2	2.0588	0.0468
8.0	8.80	4	0.00	0.00	*	0	--	--

* Asterisk indicates mean significantly less than control (Duinnett's test).

Geometric mean of survival NOEC (2.06 mg N/L) and LOEC (3.67 mg N/L) gave chronic value of 2.75 mg N/L (= unionized NH3 of 0.152 mg N/L)

A Regional Study With National Implications



125

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EPA

AQUATIC LIFE AMBIENT WATER QUALITY CRITERIA FOR AMMONIA – FRESHWATER 2011

Revision

A Regional Study With National Implications

Criterion Continuous Concentration (CCC)

Review and Analysis of Chronic Data

The freshwater final chronic value, or CCC, was updated using newly acquired chronic data for three freshwater mussel species, one freshwater non-pulmonate snail species, and three fish species (Table 5). Each chronic dataset was reviewed following the three-step review process

A Regional Study With National Implications

Table 5. Chronic Toxicity of Ammonia to Aquatic Animals.

Species	Test and Effect	pH	Temp (°C)	Total Ammonia (mg N/L)	Chronic value[a] Adjusted to pH 8 (all *organisms) and 25°C (invertebrates)* (mg N/L)	SMCV (mg N/L)	GMCV (mg N/L)	Reference
Freshwater Invertebrates (Mollusks)								
Wavy-rayed lamp mussel (2 mo old juveniles), *Lampsilis fasciola*	28-d Juv Survival	8.2	20	0.3981	<0.3917	<0.3917	<0.3443	Wang et al. 2007a
Fatmucket (2 mo old juveniles), *Lampsilis siliquoidea*	28-d Juv Survival	8.2	20	0.3076	<0.3027 (IC20)	<0.3027		Wang et al. 2007a
Rainbow mussel (2 mo old juveniles), *Villosa iris*	28-d Juv Survival	8.2	20	0.9965	<0.9805 (IC20)	<0.9805	<0.9805	Wang et al. 2007a
Long fingernail clam, *Musculium transversum*	42-d Juv Survival	8.15	23.5	5.820	6.630	<2.260	<2.260	Anderson et al. 1978
Long fingernail clam, *Musculium transversum*	42-d Juv Survival	7.8	21.8	1.230	0.7659			Sparks and Sandusky 1981
Pebblesnail (large mixed aged, field collected), *Fluminicola sp.*	28-d Juv MATC - Survival	8.20	20.1	2.75	<2.681	<2.681	<2.681	Besser et al. 2011

Pebblesnail is one of the four most sensitive genera
Only species in its genus
Included in calculation of criterion continuous concentration for ammonia
Acute-chronic ratio not used to calculate ammonia CCC

Mussel Toxicity Testing RM Proposal

Regional Methods Development Proposal on Freshwater Mussel Toxicity Testing

Title: Expansion of Freshwater Mussel Water and Sediment Toxicity Testing Methods

Regional Contact: Burt Shephard, Office of Environmental Assessment (OEA), USEPA Region 10, 1200 6th Avenue, Suite 900, Seattle, WA 98101. (206) 553-6359.

Region 10 Unit Manager: Sheila Fleming, OEA, (206) 553-1417.

Regional Division Director: Joyce Kelly, OEA, (206) 553-4029.

ORD Research Partner: ORD Lead Scientist: Teresa Norberg-King, USEPA Mid-Continent Ecology Division, 6201 Congdon Boulevard, Duluth, MN 55804-1636. (218) 529-5163

Other Research Partner: Chris Ingersoll and Ning Wang, U.S. Geological Survey, U.S. Department of the Interior, Columbia Environmental Research Center, 4200 New Haven Road, Columbia, MO 65201-8709. (573) 441-2946 (nwang@usgs.gov), 573 876-1819 (cingersoll@uusgs.gov).

Goals of Mussel Toxicity Study

- Expanding the limited number of standard freshwater toxicity test species resident to the western U.S.
- Development of methods for collecting, culturing and conducting water-only and sediment toxicity tests with freshwater mussels native to the western U.S.
- Begin the transfer of mussel water and sediment toxicity testing technology to commercial laboratories

Similarities and Differences Between a QAPP and an RM Study

Parameter	Sediment QAPP	Regional Methods
Sediment collection date	2012 first round	June 2013 - May 2014= year two June 2012 - May 2013= develop lab culture and tox testing methods with water only to evaluate relative sensitivity to a variety of PNW mussels
Number of stations	12 identified in LOE	Not specified, but will be from 3 sites, metals, PAH/HCs, and ag/insecticides. Likely 5 stations plus a reference from each of the 3 sites. Could be more stations if year 1 work comes in under budget, but can't guarantee this.
Contaminants	Metals are major concern	Water tests will include copper as the only metal (other chemicals are nonylphenol, ammonia, pentachlorophenol, carbaryl, permethrin Sediment tests will be from the UCR site for metals, also proposing to collect from PAH and insecticide/agricultural chemical sites.
Lab BLM parameters	Yes	Not specified. Likely could easily add this element with little if any budgetary impact. Will confirm with USGS
Slag measure	Yes	Not specified. Potential budget buster for the RM work unless significant cuts made to already proposed items.
Species	Hyalella and Chironomus	No Hyalella and Chironomus. Preferred test species western pearlshell (*Margaritifera falcata*). Other possibilities include *Anodonta oregonensis*, *A. californiensis*, and *A. kennerlyi*. *Lampsilis siliquoidea* as reference for comparison to other mussel species
Interlab comparison	No	Yes (June 2013 - May 2014)

Acknowledgements

- EPA-ORD: Dave Mount, Sandy Raimondo, Teresa Norberg-King
- USGS: John Besser, Chris Ingersoll, Doug Hardesty, Eugene Greer, Chris Mebane, Ning Wang, Ryan Warbritton, Rip Shively
- USFWS: Susan Burch, Michael Morse, Gary Burton, Dave Hopper


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